The Effects of Phenoxybenzamine and Cold Stress On Tyrosine Hydroxylase Enzyme Activity and Expression of Tyrosine Hydroxylase Gene in the Rat Adrenal Medulla

Muhittin YÜREKLİ
İnönü University Arts and Science Faculty Molecular Biology Department
Malatya-TURKEY

Received: 22.09.1997

Abstract: The effects of cold exposure and cold plus phenoxybenzamine treatment on tyrosine hydroxylase (TH) activity and TH mRNA levels were investigated in the adrenal medulla of Sprague Dawley (SD) rats. SD rats were exposed to cold (4 °C) for 48 hours. Phenoxybenzamine was injected intraperitoneally (i.p) at 20 mg/kg, which was prepared in 5% ethanol containing 0.9 % NaCl.

TH activity was measured by monitoring the formation of $^{3}$H$_{2}$O as a product of L-(3H)-tyrosine hydroxylation, the formation of L-(3H)-dopa from L-(3H)-tyrosine. Total adrenal RNA was isolated and blotted onto a nylon membrane and the immobilized RNA was then hybridized from TH cDNA probe. TH mRNA was assayed by densitometric scanning of the autoradiograms with a videodensitometer.

TH activity and mRNA levels were found to be significantly increased in the adrenal medulla of SD rats exposed to cold for 48 hours and treated with cold plus phenoxybenzamine (P<0.01).

Key Words: Cold exposure, adrenal medulla, phenoxybenzamine, tyrosine hydroxylase, tyrosine hydroxylase mRNA

Phenoxybenzamine ve Soğuk Stresinin Sıçan Adrenal Medulla Tirozin Hidroksilaz Enzim Aktivitesi ve Tirozin Hidroksilaz Gen Ifadesi Üzerine Etkileri

Özet: Soğuk uygulaması ve soğukta ek olarak phenoxybenzamine uygulamasının, tirozin hidroksilaz (TH) ve TH mRNA seviyeleri üzerindeki etkileri Sprague Dawley (SD) sıçanlarının adrenal medullaslarda araştırıldı. SD sıçanları 48 saat 4 °Cde soğuğa maruz bırakıldı. Phenoxybenzamine uygulaması % 0.9 NaCl içeren % 5 etanol içinde hazırlanarak 20 mg/kg olacak şekilde periton içine (i.p) yapıldı.

TH aktivitesi, L-(3H)-tirozindend L-(3H)-dopa oluşurken, L-(3H)-tirozinin hidroksilasyon ürünü olan 3H2O'nun izlennmesi ile ölçüldü. Total adrenal RNA izole edildi ve naylon membrana bağlandı, bu şekilde immobilize olan RNA daha sonra TH cDNA probu ile hibritleştirildi. TH mRNA miktarları, otoradyogramlarının videodensitometre kullanarak densitometrik taramalar yapılarak saptandı.

SD sıçanlarının adrenal medullasında TH aktivitesi ve TH mRNA seviyelerinin, 48 saat soğuk uygulaması ve soğuğa ek olarak phenoxybenzamine verilen gruplarda önemli derecede artışı saptandı (P<0.01).
Introduction

Exposure to cold and the administration of antihypertensive drugs are known to proceed the synthesis and release of catecholamines in the adrenal medulla (1-8). Tyrosine hydroxylase is the rate limiting step in the biosynthesis of catecholamines (TH; tyrosine-3-monooxygenease, EC: 1, 14, 16, 2). TH catalyses the formation of DOPA (dihidroxy-phenylalanine) from L-tyrosine (9-13). The rate of synthesis of norepinephrine and epinephrine is controlled by the activity of tyrosine hydroxylase. Catecholamines are synthesized in the adrenal medulla, hypothalamus substantia nigra, locus cereleus, putamen, striatum, cortex cerebri and neuron terminals. Experimental studies in pharmacology, physiology and biochemistry have clearly shown that catecholamines play neuroendocrine and endocrine roles in mediating a variety of autonomic functions in the periphery. Normal catecholamine function is critical for maintaining homeostasis (14). Physical activity, psychological stress, drug treatment and generalized allergic reactions enhance the synthesis and release of catecholamines in organisms. Changes in TH enzyme activities have been investigated in the periphery and adrenergic nervous system after some pharmacological and physiological treatment (15, 16).

Cold stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis, and it is important to consider the role of glucocorticoids in regulating norepinephrine function in the brain (17). Phenoxybenzamine inhibits reuptake of released norepinephrine by presynaptic adrenergic nerve terminals. The pharmacological actions of phenoxybenzamine are primarily related to antagonism of alpha-receptor mediated events. Phenoxybenzamine blocks catecholamine-induced vasoconstruction (18-27). It also increases blood flow to the skin, mucosa and abdominal viscera, and lowers both supine and erect blood pressures. It has no effect on the parasympathetic system.

The aim of this study was to compare differences in TH activity and TH mRNA levels between cold-exposed and cold+phenoxybenzamine-treated animals. In addition, the ability of the catecholaminergic system of SD rats to respond to cold exposure and cold plus phenoxybenzamine was investigated.

Materials and Methods

Ten male Sprague Dawley (SD) rats, 3 months old, were used in the present study. Rats were housed individually in cages with food and water ad libitum. The temperature was 26 °C. Five rats were maintained at 26 °C as control animals. For cold exposure, 5 rats were placed in a 4 °C chamber (Revco Cryo-frig.). Phenoxybenzamine was prepared in 5% ethanol containing 0.9% NaCl and injected in cold-exposed rats as 20 mg/kg i.p. 0.9% NaCl of 5% ethanol was injected in control animals (LD50 for phenoxybenzamine was approximately 2000 mg/kg in rats) (18, 20). animals were anaesthetized with pentobarbital and adrenal glands were quickly removed and rapidly frozen in liquid nitrogen. Tissues were stored at -80 °C until use. Tissues were homogenized in phosphate buffer (pH=7.0, 2 mM 1% triton-x) for 15 seconds in an ice bucket in the sonificator. TH activity, total protein and TH mRNA were determined in aliquots of the sample. Total protein was quantified according to Bradford (28). TH activity was meas-
ured using radioenzymatic assay, as described by Reinhard et al. (29). TH activity was measured using radioenzymatic assay, as described by Reinhard et al. (29). TH activity was determined by monitoring the formation of $^3$H$_2$O as a product of L-$[^3]$H-tyrosine hydroxylation, the formation of L-$[^3]$H-dopa from L-$[^3]$H-tyrosine. Determination of TH enzyme activity was as follows: 25 µl homogenate was analyzed at pH 7.0 in the presence of 6-MPH (DL-6 Methyl-5,6,7,8-tetrahydropterine; DL-2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine, Sigma M4758) and [3,5, $^3$H]tyrosine (100 µM, 1 µCi) (Sigma) in a total volume of 50 µl for 15 min at 37 °C. Total adrenal medullary RNA was isolated by using RNAzolB (Biotec, Friendswood, TX). Total RNA was quantified spectrophotometrically at 260 nm (4,30). Diluted RNA samples were blotted onto nylon membrane (Gene Screen, New England Nuclear, Boston, MA) using a slot blot apparatus. The filters were baked at 80 °C for 2-4 h, then prehybridized with 50 µl denatured salmon testes DNA. Following incubation for 14-16 h at 42 °C, filters were hybridized with a $^{32}$P TH.36 cDNA probe (supplied by Dr. Karen O’Malley Washington University, School of Medicine, by Dr. Nihal Tümer, University of Florida, Pharmacology Department). The resulting $^{32}$P labeled RNA-DNA hybrids were detected by autoradiography using Kodak x-ray films. TH mRNA was assayed by densitometric scanning of the autoradiograms, using a densitometer (BioRad, 620 video densitometer). The amount of TH mRNA was expressed as OD units per µg of total RNA. Means and SEMs were calculated from values obtained from a pair of adrenal medulla. Comparison of means among control and treatment groups was made by Student’s t-test.

Results and Discussion

TH activity and TH mRNA were tested in cold-exposed and phenoxybenzamine-treated animals. Table 1 gives the weight of adrenal glands, the amount of total protein and the TH enzyme activity in the control and treatment rats. Total protein and tissue weight was higher treatment and cold-exposed animals than in control animals. TH enzyme activity was 31±0.79 nmol/mg protein/h in control rats, 49.32±3.65 nmol/mg protein/h in cold-exposed rats and 63.17±3.53 nmol/mg protein/h in phenoxybenzamine-treated rats (Figure 1). Cold exposure and cold+penoxybenzamine altered TH enzyme activity, which was significantly higher (P<0.01) in the cold-exposed and treatment groups than in the control group (Table 1). TH mRNA was significantly higher in the adrenal medulla of the cold-exposed and treatment groups (P<0.01) than in that of the control group. The total RNA and TH mRNA levels are shown in Table 2.

A gradual increase in catecholamine biosynthesis may be caused by a prolonged increase in the activity of sympathetic nerves through the administration of reserpine, phenoxy-benzamine, prazosin or 6-hydroxydopamine, drugs which lower blood pressure. These drugs were found to increase the activity of tyrosine hydroxylase in the adrenal gland (7, 31). There are two explanations of the increase in TH activity and TH mRNA levels of cold-exposed and cold +plus-phenoxybenzamine-treated animals. The first is that peripheral thermoreceptors are stimulated by an increase in splanic nerve activity or the stimulation of baroreceptors. The second is that the hypothalamus-pituitary-adrenal axis and glucocorticoids play such a role.
The Effects of Phenoxybenzamine and Cold Stress On Tyrosine Hydroxylase Enzyme Activity and Expression...

Table 1. Effects of cold exposure and cold plus phenoxybenzamine treatment on TH activity in SD rats

<table>
<thead>
<tr>
<th>Tissue Wt. (mg)</th>
<th>Total protein (µg/µl)</th>
<th>TH Activity (nmol/mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.16 ±1.53</td>
<td>41.32±3.74</td>
</tr>
<tr>
<td>Cold exposed</td>
<td>30.98±1.09</td>
<td>50.53±1.83*</td>
</tr>
<tr>
<td>Cold+ Phenoxybenzamine</td>
<td>42.24±2.85**</td>
<td>44.16±2.97</td>
</tr>
</tbody>
</table>

* Significantly different with cold exposure P<0.01
** Significantly different with cold+phenoxybenzamine P<0.01

Table 2. Amount of total RNA and TH mRNA in cold exposed and cold plus phenoxybenzamine treated rats

<table>
<thead>
<tr>
<th></th>
<th>Total RN mg/ml</th>
<th>ODUnit/mg RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.90±0.13</td>
<td>0.35±0.07</td>
</tr>
<tr>
<td>Cold exposed</td>
<td>5.67±0.92*</td>
<td>3.57±0.63*</td>
</tr>
<tr>
<td>Cold+Phenoxybenzamine</td>
<td>3.36±0.24**</td>
<td>1.31±0.18**</td>
</tr>
</tbody>
</table>

*Significantly different with cold exposure P<0.01
**Significantly different with phenoxybenzamine P<0.01

Figure 1. The effects of cold exposure and cold plus phenoxybenzamine treatment on the adrenal TH activity of SD rats.

A similarity is found in the increase of TH activity and TH mRNA levels when treated animals are compared with controls. The rate of TH activity was 1.5-fold in cold-exposed and 2-fold in cold-plus-phenoxybenzamine-treated rats. Furthermore, TH mRNA levels were 10-and-3.5 fold in cold-exposed and cold+phenoxybenzamine-treated animals, respectively (Figure 2).
It has been shown that an increase in TH activity depends on increased TH mRNA levels following administration of reserpine or exposure to cold (5, 38, 33). Sharma and Khanna have reported increased catecholamine levels following cold stress. This is the result of hypothalamic stimulation (34). Stachowiak et al. have reported that cold stress increases TH enzyme activity in catecholamine biosynthesis (33). Baruchin et al. have shown that rats subjected to 5 °C cold exposure for 1 hour had maximal increases of 300-400 %, levels that the controls took 3-6 hours to reach, and that levels remained constant for the duration of cold exposure (35).

These findings provide a molecular approach to the adaptation of the catecholaminergic system during cold stress and administration of drugs. Our results suggest this phenomenon.

Organisms may be exposed to more severe stressors during their lives, for cold stress, hard exercise, drug treatment and physiologic stress. Further investigations are required to understand the underlying mechanisms directing the catecholaminergic system of organisms for responding and adapting to more than two stressors.

**Acknowledgments**

This study was conducted at the Geriatric Research Education and Clinical Center of Veterans Affairs, Florida, USA. We thank Pharmacologist Dr. Nihal Tümer for advising and providing us with TH cDNA probe.
The Effects of Phenoxybenzamine and Cold Stress On Tyrosine Hydroxylase Enzyme Activity and Expression...

References


